B-5494
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RESEARCH ARTICLE Indian Journal of Animal Research

Pathomorphology of Mycoplasmal Pneumonia Associated with *Mycoplasma ovipneumoniae* Infection in an Organized Goat Farm

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10.18805/IJAR.B-5494

ABSTRACT

Background: Mycoplasmal pneumonia causes severe economic loss in goat farms due to loss of production performance and mortality. *Mycoplasma ovipneumoniae* is an important pathogen causing mycoplasmal pneumonia in small ruminants. The present research work was carried out to study the pathomorphological alterations associated with *M. ovipneumoniae* infection in an organized goat farm.

Methods: The research work was carried out in an organized goat farm having history of respiratory illness with mortality and suspected for mycoplasmal pneumonia. Samples from ailing animals and dead animals were collected for investigation. Tissue samples from lungs, trachea, mediastinal lymph nodes and swabs from lungs, trachea and pleural fluid were collected during postmortem examination and subjected to laboratory examination.

Result: The samples were confirmed to be positive for *M. ovipneumoniae* infection by PCR using specific primers. The clinical signs of *M. ovipneumoniae* infection observed were anorexia, mucopurulent nasal discharge, coughing, increased body temperature, respiratory distress and death in severely affected animals. In the present study, 10 animals exhibited clinical signs and 3 animals died out of 10 animals. Necropsy lesions observed in lungs were fibrinous adhesions, severe consolidation of left lung and blackish discoloration of right lung. Histopathologically, lesions in lungs were characterized by widespread congestion, presence of inflammatory cells, widened interlobular septa with fibrin and inflammatory cells. Congestion of bronchioles and presence of pinkish fibrinous exudate within the bronchiolar lumen were observed. From the present study it is concluded that *M. ovipneumoniae* infection causes severe pathology of respiratory organs and mortality in goats under stressful conditions like long transport.

Key words: Goats, *Mycoplasma*, Mycoplasmosis, *Ovipneumoniae*, Pathomorphology.

INTRODUCTION

Goat farming plays an important role in the upliftment of economic status of rural farmers and provides dependable source of income especially for the small and marginal farmers in India. According to 20th Livestock Census (2019), India has 74.26 million numbers of sheep and 148.88 million numbers of goats.Respiratory tract diseases are the most important cause of mortality in goats. Pneumonia is considered to be the single largest cause of death in sheep and goats and is responsible for around 28.70 per cent mortality (Parthiban *et al*, 2020). Among the important respiratory diseases of goats, mycoplasmal infections result in significant economic losses (Yatoo *et al.,* 2018). Mycoplasmosis in goats causes severe economic loss due to loss of production performance and the cost incurred towards treatment and control of the disease. Recently mycoplasmosis is becoming an emerging threat and transboundary epidemiological disease posing a worldwide regulation on goat production and it results in huge economic loss to the farmers (Manimaran *et al.,* 2020).

Mycoplasmas are the smallest self-replicating prokaryotes measuring 300-800 nm diameter, having no cell wall but are bounded by a plasma membrane. The most important mycoplasmas of goats are pathogens of the *Mycoplasma mycoides* cluster which is composed of six genetically and immunologically similar *Mycoplasma* spp. Contagious caprine pleuro pneumonia (CCPP) caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp/ previously known as *Mycoplasma* biotype F38) is the most

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How to cite this article: Kohila, P., Balachandran, P., Balasubramaniam, G.A., Balasubramaniam, A., Selvaraju, G., Arulmozhi, A., Srinivasan, P., Sasikala, M. and Gopal, K. (2025). Pathomorphology of Mycoplasmal Pneumonia Associated with *Mycoplasma ovipneumoniae* Infection in an Organized Goat Farm. Indian Journal of Animal Research. 1-7. doi: 10.18805/IJAR.B-5494.

Submitted: 20-09-2024 **Accepted:** 19-02-2025 **Online:** 30-06-2025

important of mycoplasmal diseases. *Mycoplasma mycoides* subsp. *capri* (Mmc) and *Mycoplasma capricolum* subsp. *capricolum* (Mcc) causes pleuropneumonia in goats (Yatoo *et al.,* 2018). Although Mccp has been reported to be the important cause of mycoplasmal pneumonia in sheep and goats, *Mycoplasma ovipneumoniae* is also reported to be an important pathogen causing mycoplasmal disease in small ruminants worldwide (Nicholas *et al.,* 2008). *M. ovipneumoniae* is a well-established respiratory pathogen of sheep and goats which is gaining more importance in recent times. Apart from detection in sheep and goats it is gaining importance in wild ruminants including members of the Cervidae family (Maksimovic *et al.,* 2022).

Mycoplasma ovipneumoniae

Although *M. ovipneumoniae* has been found in respiratory tracts of apparently healthy sheep and goats, it has been frequently isolated from cases of mycoplasmal pneumonia. The organism has been reported to cause heavy economic loss mostly due to poor growth rate and productivity loss in sheep and goats (Besser *et al.,* 2019). Respiratory infections caused by *M. ovipneumoniae* are known by various names like mycoplasma pneumonia, atypical pneumonia of sheep, chronic bronchopneumonia, chronic non progressive pneumonia and proliferating exudative pneumonia (Sheehan *et al.,* 2007 and Goncalves *et al.,* 2010). The disease is known as summer pneumonia in Australia and New Zealand due to increased prevalence of the disease in hot climatic conditions (Alley *et al.,* 1999).

The organism is mainly transmitted *via* the respiratory route following close with the infected animals. *M. ovipneumoniae* infection can cause variable morbidity and low mortality. Presence of strains with variable virulence, presence of co-infections and the host immune response plays an important role in the outcome and severity of disease. It is the common predisposing factor for other bacterial pneumonia, particularly pasteurellosis and viral infections, which may intensify the pathological process (Nicholas *et al.,* 2008). In sheep and goats, within the flocks' multiple strains of *M. ovipneumoniae* have been found and in single animal, presence of multiple strains leads to more severe disease. But in several reports *M. ovipneumoniae* has been found to be the sole cause of mycoplasmal pneumonia in goats (Rifatbegovic *et al.,* 2011). *Mycoplasma* is a fastidious organism and it is very difficult to isolate on culture media *in vitro*.The PCR assays based on amplification of 16S rRNA gene of the *mycoides* cluster can be used for confirmation of mycoplasmosis (Bascunana *et al.,* 1994). Manimaran *et al.* (2020) reported that, PCR is a reliable tool for early detection of the disease condition and more rapid and accurate method for identification of caprine mycoplasmosis from clinical specimens than conventional culture methods.

In view of these, the present research work was carried out to study the pathomorphological alterations associated with *M. ovipneumoniae* in an organized goat farm.

MATERIALS AND METHODS

The present research work was carried out during the period from July 2022 to June 2024 in the Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu to study the patho-mophology of mycoplasmal pneumonia in goats. During the study, an outbreak of mycoplasmal pneumonia was recorded in an organized goat farm in Namakkal district of Tamil Nadu. The flock size was 75 with 40 numbers of Tellicherry goats and 35 numbers of crossbred Boer goats. The outbreak occurred after introduction of new animals into the already existing flock of Tellicherry goats. Out of the total 40 Tellicherry goats, 10 animals exhibited clinical signs of respiratory infection. Out of the 10 affected animals, 3 animals died over a period of 10 days in spite of treatment with antibiotics and other medications. Postmortem examination was conducted and samples were collected for laboratory examination. The tissue samples including lungs (n=3), trachea (n=3), mediastinal lymph nodes (n=3), lung swab (n=3), tracheal swab (n=3) and swabs from pleural fluid (n=3) were collected aseptically and immediately stored at -20°C until further analysis. Tissue samples from lungs, trachea and mediastinal lymph nodes were collected in 10% neutral buffered formalin for histopathological examination. Nasal swabs (n=6) were also collected from the ailing animals of the flock for molecular diagnosis.

Polymerase chain reaction for confirmation of *Mycoplasma* genus and *M. ovipneumoniae* spp.

As it is very difficult to isolate *Mycoplasma* spp. on culture media due to its fastidious nature, PCR was carried out for confirmation of the disease. PCR was reported to be the rapid, sensitive and specific test for confirmation of caprine mycoplasmosis (Manimaran and Singh, 2018). In this study, DNA extraction was carried out from swab and tissue samples. For swab samples 400 µl of normal saline was added to the tubes, shaked in vortex mixture, from this 200 µl of sample was taken and used for DNA extraction using Amplicon QIAmp mini kit. For tissue samples 1 gram of tissue sample was ground in pestle and mortar with 400 µl of normal saline. The sample was mixed in vortex mixture and from this 200 µl of sample was taken and used for DNA extraction.

20 µl of reaction mix was prepared each containing 10 µl master mix (Amplicon), 1 µl each of forward and reverse primer, 2 µl of sample DNA and 6 µl of nuclease free water. Amplification was done in gradient thermocycler and amplified products run in electrophoresis using 1.5% agarose gel stained with ethidium bromide at 80 volts, 400 mA for 45 minutes. PCR products with 715 bp were considered positive for *Mycoplasma* genus and PCR products with 418 bp were considered positive for *M. ovipneumoniae* species (Table 1).

Histopathology

Histopathological processing of tissues was carried out as per the method described by Bancroft *et al.* (2008).

Tissues in paraffin embedded blocks were prepared, thin sections of 3 μm thickness were cut and stained with haematoxyin and eosin for histopathological examination.

RESULTS AND DISCUSSION

In the present study, the outbreak was recorded soon after the introduction of new animals into the existing farm and 3 animals died out of affected 10 animals. The outbreak had occurred due to transport of animals for a long distance that created stress and favoured multiplication of organisms and subsequently resulted in clinical disease. Poor climatic conditions, transport of animals to a long distance and reduced level of immunity in the flock are the predisposing factors for development of mycoplasmal pneumonia in goats (WOAH, 2008). In mycoplasmosis various factors such as intercurrent infections, crowding, stress from transportation, age and genetic constitution are important determinants of the final outcome of infection (Parthiban *et al.,* 2020). In India, caprine mycoplasmosis has been reported in many states including Andhra Pradesh, Assam, Goa, Gujarat, Himachal Pradesh, Kerala, Maharashtra, Rajasthan and Tamil Nadu (Parthiban*et al.,* 2020). Clinical outbreaks of mycoplasmosis in goats especially CCPP in a flock often results in 80.00 % morbidity and mortality rate of 62.00 % in untreated cases (Abraham *et al.,* 2015). Erythromycin and tylosin followed by tetracycline and doxycycline were found to be the drugs of choice for caprine mycoplasmosis (Kalmegh *et al.,* 2020). Concurrent infections of *Mycoplasma* spp. with *E. coli*, *P. multocida* and *Staphylococcus* spp. were reported from pneumonic cases of small ruminants (Babu, 2016). Concurrent infections with viral disease such as Peste des Petits Ruminants (PPR) predispose the lung tissue for invasion by *Mycoplasma* spp. In goats, concurrent diseases such as PPR, Mycoplasmosis and Pasteurellosis were reported by Shanmugavadivu *et al.* (2021).

Jay *et al.* (2020) reported that, among the total *Mycoplasma* spp. isolates from small ruminants 16.4% of isolates were identified as *M. ovipneumoniae*. In a study by Deeney *et al.* (2021), *M. ovipneumoniae* isolates constituted more than half of all mycoplasmas isolated in small ruminants in England. Manlove *et al.* (2019) reported that *M. ovipneumoniae* was detected in 88% of 453 domestic sheep operations across USA. A study in China revealed that the seroprevalence of *M. ovipneumoniae* was 18 % and PCR detection rate was 10% in nasal swabs and 30% in lungs (Cheng *et al.,* 2015).

Molecular diagnosis

The presence of *Mycoplasma* Spp. in postmortem samples and nasal swab of ailing animals was confirmed by PCR. *Mycoplasma* genus was confirmed based on PCR products of 715 bp (Fig 1) visualized in 1.5 % agarose gel electrophoresis and *M. ovipneumoniae* was confirmed based on PCR products of 418 bp (Fig 2). Similar findings were also reported by Halium *et al.* (2019) and Manimaran *et al.* (2022).

Clinical signs

The clinical signs of *M. ovipneumoniae* infection in the present study were increased body temperature, anorexia, mucopurulent nasal discharge, coughing, respiratory distress and death in severely affected animals.

In *M. ovipneumoniae* infection, clinical signs may be mild to moderate in which cases the animals may recover or in some cases pneumonia may persists for longer duration. Similar clinical signs like nasal discharge, coughing, rise in temperature, increased respiratory rates, reduced appetite and growth rate were also reported by Ayling *et al.* (2007). Experimental infection in lambs by *M. mycoides* ssp. *mycoides* (LC) and *P. hemolytica* resulted in clinical signs including rise in temperature, ocular and nasal discharge, dullness, coughing, sneezing, arching of back, laboured breathing and blood tinged nasal discharge in terminal stages of disease (Batra *et al.,* 2003).

Gross pathology

Gross lesions observed in the present study were presence of serosanguinous fluid and diffuse fibrin deposition in the thoracic cavity. Apical and cardiac lobes of left lung had severe fibrin deposition and attached to the chest wall. Severe consolidation of cardiac, apical and part of diaphragmatic lobes of left lung was observed. Right lung showed consolidation, blackish discoloration and was leathery in appearance and texture. The lesions in heart

Table 1: Sequence of primers used for amplification (Halium *et al.,* 2019 and Manimaran *et al.,* 2022).

PCR	Primer	Amplicon base size	PCR conditions
Mycoplasma group specific PCR	Forward primer: GPO-1: 5′-ACT CCT ACG GGA GGC AGC AGT A-3′ Reverse primer: MGSO : 5′ -TGC ACC ATC TGT CAC TCT GTT AAC CTC-3′	715 bp	· Initial denaturation: 5 min. at 94°C · 35 cycles of; Denaturation:1 min at 94°C Annealing: 1 min. at 55°C Extension: 1 min. at 72°C · Final extension :2 min. at 72°C
Mycoplasma ovipneumoniae specific PCR:	Forward primer: MOVPF: 5′ GTT GGT GGC AAA AGT CAC TAG 3′ Reverse primer : MOVPR: 5′ CTT GACATC ACT GTT TCG CTG 3′	418 bp	· Initial denaturation: 5 min. at 94°C · 35 cycles of;Denaturation: 1 min. at 94°C Annealing: 90 sec. at. 62°C Extension: 1 min. at 72°C · Final extension: 2 min. at 72°C

were thickened pericardial sac, presence of serosanguinous fluid and fibrin attached to visceral surface of pericardium (Fig 3). Cut section of the lungs revealed the consolidated areas and presence of frothy serosanguinous exudate in bronchioles. Trachea contained frothy exudates. Edema and congestion of mediastinal lymph nodes was also noticed.

Mondal *et al.* (2004) reported that, the lesions in caprine mycoplasmal pneumonia were characterized by unilateral or bilateral involvement of lung along with lymph node enlargement and serosanguineous strands in pericardium and peritoneum. Congested trachea, frothy exudates and chronic tracheitis were usually observed. Acute disease was characterized by unilateral pneumonia and serofibrinous pleuritis with straw coloured fluid in the thorax. Cut surface of lung was granular with copious straw-coloured exudates. Pea-sized, yellow nodules may be found in the lungs and the nodules may be surrounded by areas of congestion. Varying degrees of lung consolidation or necrosis can be seen with enlarged bronchial lymphnodes (WOAH, 2008). In CCPP, fibrinous pleuropneumonia with massive unilateral lung hepatization and accumulation of straw-coloured pleural fluid were observed. In some cases, the pleural exudates got solidified and formed a gelatinous covering over the whole lung (Parthiban *et al.,* 2020). The lesions in concurrent infection of CCPP and PPR were extensive pleuritis with large fibrin clots on lung surface and greyish pink consolidation of cranial lobes and anterior parts of diaphragmatic lobes. Cut section of lungs revealed granular appearance of consolidated areas. Mediastinal lymph nodes were edematous and congested (Shanmugavadivu *et al.,* 2021).

In *M. ovipneumoniae* infection, pneumonia may be more severe when it is accompanied with stress and it results in acute fibrinous pneumonia. The lesions observed in the present study concur with the findings of Alley *et al.* (1999) who observed the postmortem lesions like lung consolidation, pleurisy and pulmonary abscess. Initially the lung lesions appeared as dull red areas of collapse accompanied by bronchiolitis in associated airways followed by lesions of firm, consolidated red-grey areas and localized pleural adhesions.

Pavone *et al.* (2023) reported that, the severity of lesions in *M. ovipneumoniae* infection in sheep and goats were almost similar. The gross lesions in lungs of sheep and goats were dark red to grey pink areas of consolidation in cranioventral lobes with occasional extension to the cranial area of caudal lobes. On the cut surface, an apparent thickening of the bronchial wall with catarrhal to purulent exudate was detected in some cases. The pleural surfaces of affected lungs were covered by abundant fibrin in occasional cases. Fibrinous pleuropneumonia and pleural adhesions reported in the present study concurs with the findings of Chen *et al*, (2024) who also reported the gross lesions like increased pleural fluid, fibrinous pleuropneumonia and localized pleural adhesions in goats died of *M. ovipneumoniae* infection.

Histopathology

Histopathologically, widespread alveolar congestion was observed in lungs and the alveoli in peribronchiolar area were the most affected one. Severe infiltration of inflammatory cells including neutrophils and lymphocytes (Fig 4) was noticed in alveolar lumen as well as in the interstitial space. Distended interlobular septa with fibrin and inflammatory cells were observed (Fig 5). Congestion of bronchioles and presence of pinkish fibrinous exudate along with inflammatory cells was observed within the bronchiolar lumen. Desquamation of tracheal mucoasal epithelium and submucosal hemorrhage were observed. The changes in the mediastinal lymph nodes were congestion and lymphoid depletion (Fig 6).

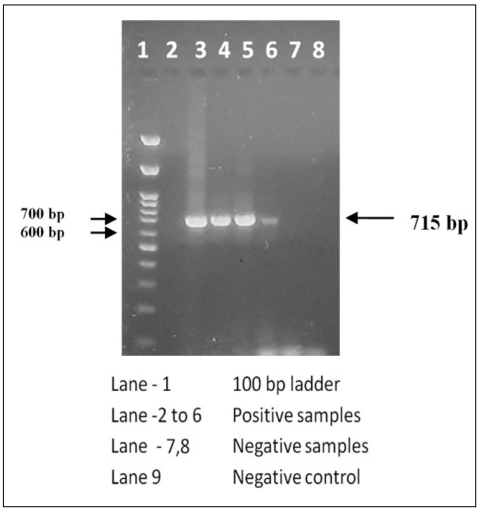

Fig 1: PCR - *Mycoplasma* spp.715 bp on agarose gel electrophoresis.

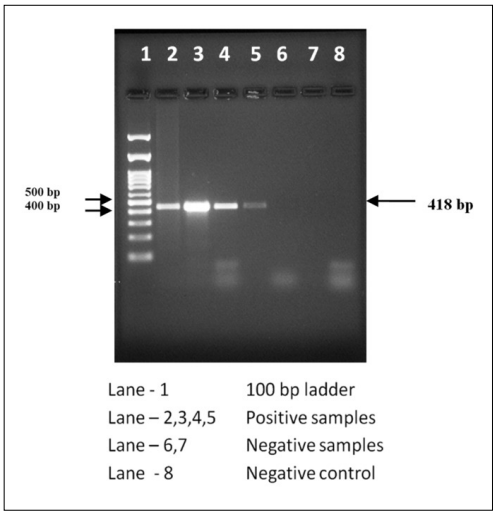

Fig 2: PCR - *Mycoplasma ovipneumoniae* - 418 bp on agarose gel electrophoresis.

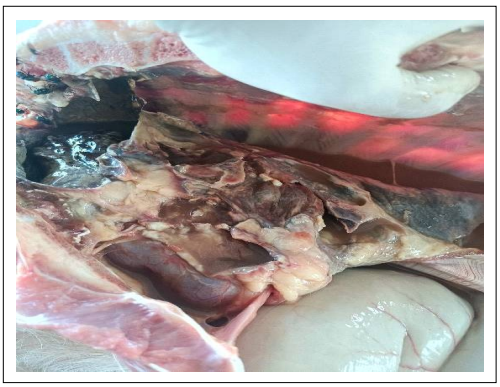

Fig 3: Thoracic cavity showing presence of serosanguinous fluid, fibrin deposition and leathery appearance of right lung.

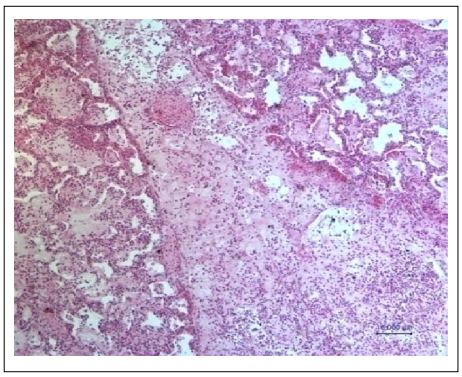

Fig 5: Lungs showing distended interlobular septa with fibrin and inflammatory cells infiltration.

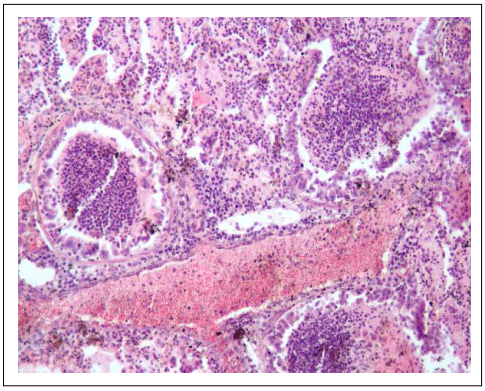

Fig 4: Lungs showing congestion and presence of polymorphonuclear cells and lymphocytes.

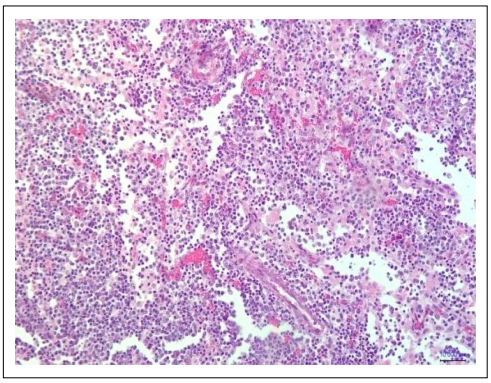

Fig 6: Mediastinal lymph node showing congestion and lymphoid depletion.

Mondal *et al.* (2004) reported that, the lesions in caprine mycoplasmosis caused by Mccp were limited to the respiratory tract only. Haemorrhage, necrosis of lining cells, infiltration of neutrophils, lymphocytes and macrophages were observed in blood vessels of the lungs. Thrombosis in lungs was constantly associated with interstitial pneumonia, atelectasis and emphysema. In trachea, erosion of the superficial layer, oedema in the muscular layer and hyperactivity of the mucous secreting cells were observed (Mondal *et al.,* 2004). Hernandez *et al.* (2006) reported that the findings were consistent with proliferative interstitial pneumonia. Thickening of the interlobular septa, presence of fibrin, increased numbers of mononuclear cells and marked vascular response characterized by congestion and hemorrhage were observed. In all instances, proliferation of the bronchus-associated lymphoid tissue (BALT) and alveolar macrophage activation was observed. The histopathological lesions in peracute to chronic cases were serofibrinous pleuro-bronchopneumonia and prominent peribronchiolar lymphoid tissue cuffing in lungs (Manimaran*et al.,* 2022). In concurrent infection of CCPP and PPR, thickening of pleura, interlobular septa and alveolar wall due to fibrin deposition and infiltration of inflammatory cells were observed. Alveolar lumen contained serosanguinous exudates with neutrophils and mononuclear cells (Shanmugavadivu *et al.,* 2021).

Histopathologically, the lesions in goats affected with *M. ovipneumoniae* were inflammatory cell infiltration and alveolar septa thickening in 97% of cases and alveolar/ bronchiolar neutrophilic exudates in 71% of cases. Septal fibrosis, fibrin exudates, bronchiolar epithelial hyperplasia, presence of alveolar syncytial cells and peribronchial lymphoid hyperplasia/lymphoid cuffing were also observed. Occasionally necrosis and thrombosis were observed (Pavone *et al.,* 2023).

CONCLUSION

From the present study it is concluded that, *M. ovipneumoniae* infection can cause severe mycoplasmal pneumonia in goats characterized by consolidation of lung, distended interlobular septa with fibrin and inflammatory cells and death in severely affected cases.

ACKNOWLEDGEMENT

The authors are thankful to Tamil Nadu Veterinary and Animal Sciences University, Chennai and Veterinary College and Research Institute, Namakkal for conducting this study.

Conflict of interest

All authors declared that there is no conflict of interest.

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